

THE CONTROLLING ELEMENT IN SEX CHROMOSOME BEHAVIOR IN SCIARA¹

HELEN V. CROUSE

Department of Botany, Columbia University, New York, New York

Received May 23, 1960

GENETIC and cytological studies have been made on a number of species of *Sciara* (see METZ 1938 for review). Common to all of these species is a series of unusual and remarkable cytological phenomena which involve the sex chromosome and which are related to the differentiation of sex in this genus. The first of these is encountered at the second meiotic division in the male, when, following the selective elimination of paternal homologues at the first spermatocyte division, the maternally derived X chromosome undergoes equational non-disjunction. Thereby, instead of a haploid set of chromosomes, such as the egg transmits (three autosomes and one X), the sperm nucleus comes to include two identical X's and three autosomes. Although the sperm transmits two X chromosomes to the zygote, in no cell of the developing embryo are both of them retained permanently: one is eliminated from the somatic nuclei of the females, both from the somatic nuclei of males, and one from the germ cells of each sex.

This pattern of sex chromosome behavior constitutes a unique sex determining mechanism. All zygotes begin development under the influence of the same chromosome complement, three X's and three pairs of autosomes. A differential between male and female is not established until anaphase of the seventh or eighth cleavage—and then only in the somatic nuclei—at which time there is an elimination of one or of both paternal X's in the production of female and male embryos, respectively (Du Bois 1933). The germ line retains all three sex chromosomes until the germ cells have migrated to the definitive gonad site; then, in both sexes alike, one paternal X is eliminated. This elimination occurs not at anaphase but in resting cells at a stage when the chromosomes are in the form of fairly compact prochromosomes; one of the paternal X's appears to migrate directly through the nuclear membrane into the cytoplasm and subsequently degenerates (BERRY 1941).

This series of interrelated and unusual phenomena demonstrates that the cells of the developing *Sciara* embryo can distinguish between sex chromosomes and autosomes, between maternal and paternal homologues. The nature of this "recognition" of chromosomes is not understood. One of the first questions to be answered, however, and one which is experimentally feasible, is whether a local-

¹ The studies reported here were supported by grants G-6176 and G-9682 from the National Science Foundation. The studies on the Oak Ridge translocations were initiated in the Biology Division of the Oak Ridge National Laboratory during the tenure of a research participantship (1957-58) sponsored by the Oak Ridge Institute of Nuclear Studies.

ized segment on the sex chromosome, or the chromosome as a whole, is involved in the process.

With this question in mind, a number of reciprocal translocations between chromosome X and the autosomes have been obtained and a study made of their inheritance and their cytological behavior during spermatogenesis (CROUSE 1943, 1960). The purpose of the present paper is to describe a new translocation in *S. coprophila*, designated "Oak Ridge T1," with which it has been possible to identify a proximal segment of heterochromatin on the X as the essential element controlling the unique behavior of this chromosome. The effects produced by the new translocation will be compared and contrasted to effects produced by a series of X translocations in the same species.

MATERIALS AND METHODS

The translocations were induced by X-ray irradiation (4000r) of sperm from monogenic strains of *S. coprophila* obtained originally from PROFESSOR METZ's

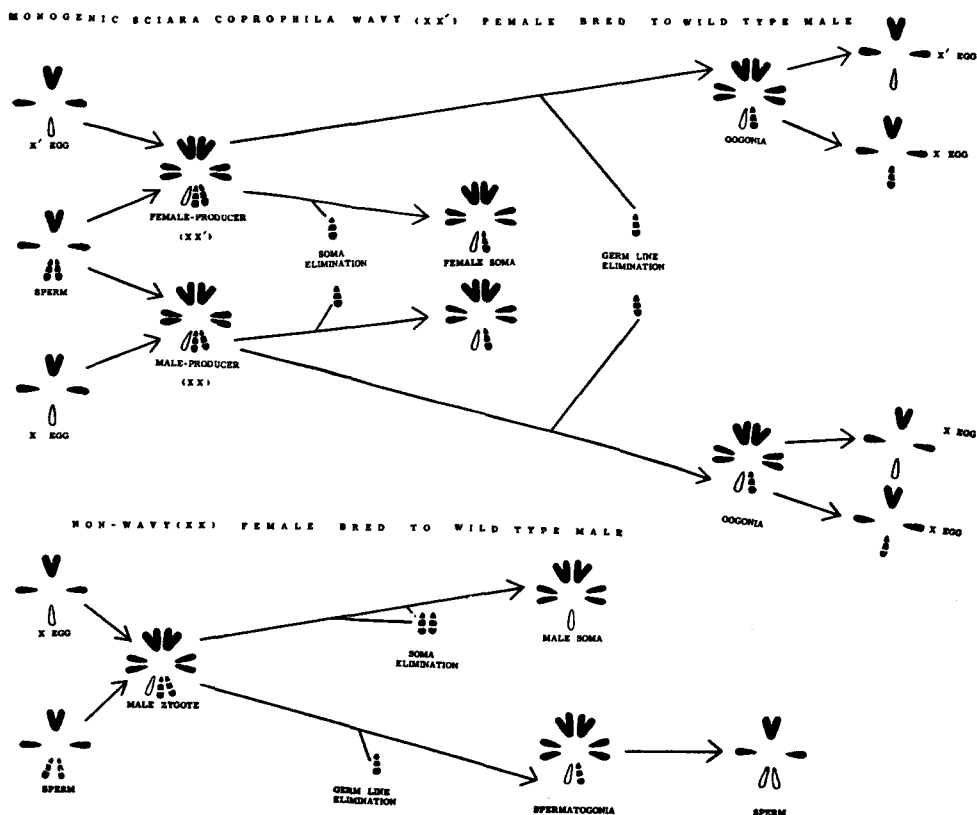


FIGURE 1.—Diagram illustrating the history of chromosomes in monogenic *S. coprophila* when Wavy females (X[']) and non-Wavy females (XX) are bred to wild type males. The limited chromosomes are omitted from consideration.

laboratory. Use was made of the available sex-linked markers, Wavy (*W*) and swollen (*sw*) and of several autosomal dominants. In contrast to digenic species of *Sciara*, in which single families consist of both sons and daughters, the females in monogenic lines are either female producers or male producers. The inheritance of sex of progeny is shown in Figure 1. Genetically there are two kinds of sex chromosomes, designated *X* and *X'*; females are either *X'X* (female producers) or *XX* (male producers), and males are normally *XX* (*X0* in soma). Wavy is a dominant marker on *X'*, and swollen is a recessive on *X*. In the earlier experiments (CROUSE 1943) the translocations were detected by means of "linkage" of autosomal and sex-linked factors. Later a more useful criterion presented itself: the occurrence, among the progeny of females heterozygous for any one of the *X* translocations, of exceptional patroclinous sons or exceptional matroclinous daughters (see CROUSE 1960).

Once an *X* translocation had been detected and the fact established that it was transmissible through both the male and female germ line, the positions of breakage on the chromosomes were determined by salivary gland chromosome analysis, and cytological studies were made on spermatogenesis in males receiving the translocation from their mother. It should be recalled from the introductory statement to this paper that, unlike oögenesis which is perfectly orthodox in *Sciara*, spermatogenesis is very unusual: each primary spermatocyte gives rise to only one sperm by virtue of the unequal nature of both meiotic divisions. At the first division there is no synapsis or crossing over, and at anaphase a directed segregation of homologues occurs as the paternally derived set of chromosomes is cast away in a bud. At the second division the maternal homologues divide equationally, but, as pointed out above, the two halves of the *X* fail to separate and both become included in the sperm nucleus. Their fate at the time of chromosome elimination in the embryo has already been described; the somatic and germ line chromosome eliminations are indicated on the diagram in Figure 1. For cytological study of spermatogenesis aceto-orcein squashes of Carnoy-fixed pupal testes were used chiefly, but these were supplemented by Feulgen-stained sections of comparable material preserved with a variety of fixatives, including Kahle, San Felice, and Gilson's corrosive sublimate.

As soon as spermatogenesis had been studied cytologically for a given translocation, the chromosome constitution of the sperm was known. With this knowledge it was possible to learn how the translocation chromosomes behaved at the "critical" stages in the embryo without the exacting cytological task of observing the actual chromosome eliminations: for, upon union of the "known" sperm with eggs produced by normal females, the nature of the somatic and germ line eliminations could be *deduced*, provided a determination was made of the somatic (salivary gland) complement and the germ line constitution (both genetic and cytological studies) of these individuals. Such genetic and cytological studies were made for nine different *X* translocations. It is not the purpose of this paper to present all of these data; some of the results have already been published, and others are in the process of publication. Included in the present paper are selected data which serve to show that the proximal mass of heterochromatin on the

X is the essential controlling element in the extraordinary behavior of this chromosome.

RESULTS

The chromosomes in the translocation material: Shown on the diagram in Figure 2 are the important structural features of the X chromosome of *S. coprophila* as observed in the salivary gland nuclei. The 36 units of length assigned to it are based on the published maps (CROUSE 1943). There are 12 map sections, each with subdivisions A, B, and C. The photomicrograph in Figure 3 shows the entire salivary complement. The exact location of centromeres on these chromosomes cannot be determined by microscopic study because there is no chromocenter, and the centric regions are not characterized by any visible differential quality. By determining the positions of chromosome breakage in 18 different translocations and the metaphase configuration for certain of these, it was possible to deduce that salivary element IV corresponds to the only V-shaped chromosome of the complement and that the proximal ends of the three remaining chromosomes are as indicated in Figure 3a (CROUSE 1943 and unpublished). Moreover, it has been possible to localize fairly accurately the X centromere in map region 12C immediately adjacent to the large mass of heterochromatin which terminates this map section and which likewise constitutes the end of the chromosome. Of key significance in this localization were translocation "Oak Ridge T1" and a long paracentric inversion on the X. The paracentric nature of the inversion was established by cytological studies (unpublished) on oögenesis comparable to those made by CARSON on *S. impatiens* (CARSON 1946). In addition to the centromere and terminal mass of heterochromatin, a third structural feature is indicated on the diagram, namely, three repeats located in map regions 2C, 10B, and 12B. These repeats are very short, each composed of a doublet and a singlet. Usually in smear preparations the interstitial one (10B) does not reveal itself through lateral synapsis (see Figure 3). It is not that this repeat fails to undergo synapsis with the other two, but that in so doing the chromosome loop produced by the segment 10B-12B is so short that the chromosome breaks in this region or the lateral attachment is ruptured. All three repeats are laterally synapsed in the cell shown in Figure 4. Indicated by means of arrows on the diagram are the positions of breakage for each of the nine translocations under

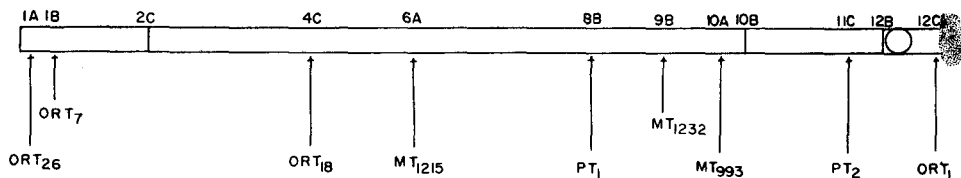


FIGURE 2.—Diagrammatic representation of the salivary X chromosome of *S. coprophila*. The centromere is shown as a circle, the terminal heterochromatin as a stippled mass. The positions of the three repeats are indicated with a vertical line and the positions of breakage for the nine translocations by arrows; the positions are given in terms of the salivary maps.

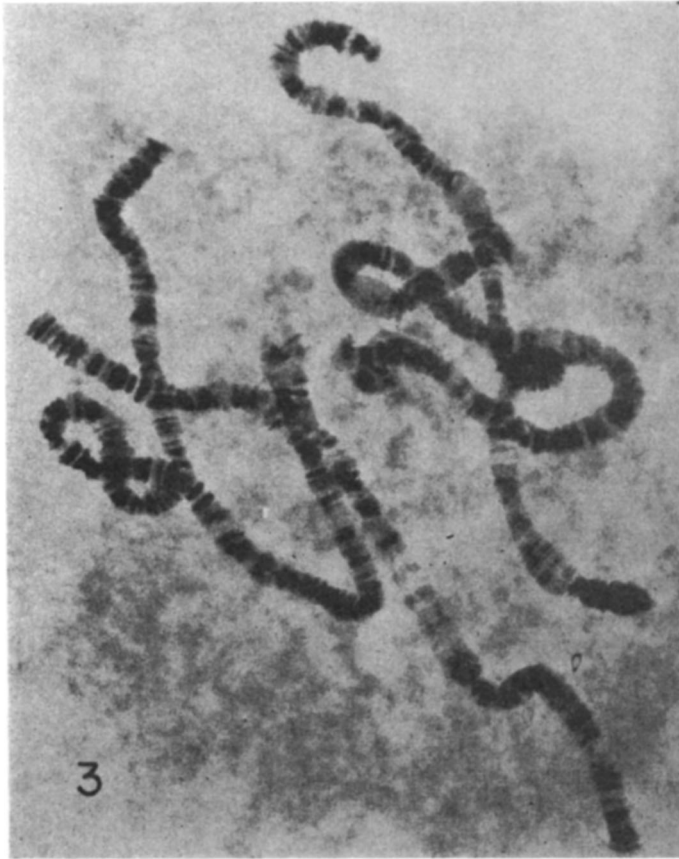


FIGURE 3.—Photomicrograph of the normal salivary complement of *S. coprophila*. $\times 600$.

study. The letters M, P, and OR designate Missouri, Pennsylvania, and Oak Ridge, respectively. Thus, "OR T1" means the first of a series of X translocations obtained at the Oak Ridge Laboratory, etc.

The spermatogenesis studies: The results of the cytological studies on spermatogenesis are summarized in Table 1. In the second column of the table the autosome involved in each of the translocations is listed, together with the position of breakage according to the salivary maps. It will be noted that all of the translocations except M T1215 were simple, involving a single break on the X and a single break on one of the autosomes. Even M T1215 was simple as regards the X breakage, and only two translocation chromosomes composed partially of X were formed by the rearrangement. Thus, in all nine cases the X was distributed between two rearranged chromosomes.

In the third and fourth columns of the table there is presented the essential behavior of the rearranged chromosomes during the crucial secondary spermatocyte division. (For the sake of brevity in the table the chromosome observed to undergo equational nondisjunction is referred to as the "precocious chromo-

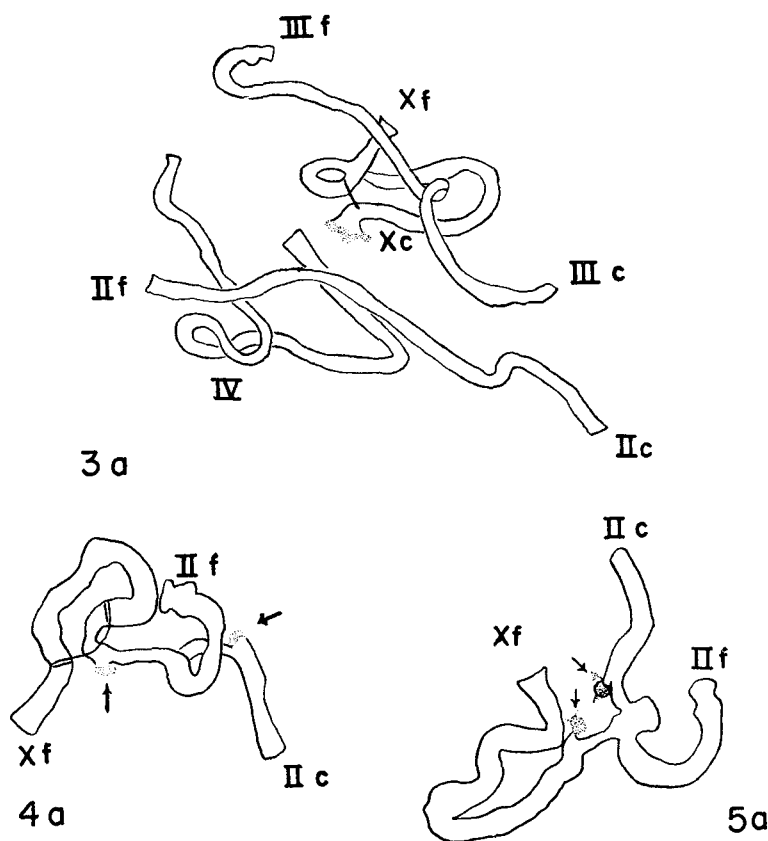


FIGURE 3a.—Outline drawing of the chromosomes shown in Figure 3. The proximal ends of the rods are indicated by the letter c, the distal ends by f. IV is the V-shaped chromosome.

FIGURE 4a.—Outline drawing of the chromosomes shown in Figure 4. The terminal heterochromatin of the X is stippled; the arrows indicate its position on the normal X and also on the translocation chromosome bearing the centromere of II.

FIGURE 5a.—Outline drawing of chromosomes shown in Figure 5.

some," the original terminology applied by METZ. For all nine of the translocations only one of the rearranged chromosomes underwent equational nondisjunction; the other chromosome underwent congression and equational division along with the autosomes. In seven of the nine cases the chromosome which displayed behavior normally characteristic of the X, i.e., equational nondisjunction, could be identified (see fifth column of the table); in all except OR T1 it was the translocation chromosome composed of a distal autosomal segment and a proximal segment of X including the X centromere and terminal heterochromatin mass.

In OR T1 the break on the X separated this terminal heterochromatin from the remainder of the chromosome. Thereby, following reciprocal exchange with the broken II, two translocation chromosomes were formed: one composed of a

TABLE 1

Cytological studies of spermatogenesis

Translocation	Autosome and break position	Shape of "precocious" chr.	Shape of other translocation chromosome	Identification "precocious" chromosome
M T993	II-18B	rod	rod	no
M T1215	II-15C	rod	V-shaped	yes
	IV-41B and 50A			
M T1232	III-25C	rod	rod	yes
P T1	II-18B	rod	rod	no
P T2	II-16C	rod	rod	yes
OR T1	II-18C	rod	V-shaped	yes
OR T7	IV-36B	rod	J-shaped	yes
OR T18	IV-49C	rod	J-shaped	yes
OR T26	IV-37B	rod	J-shaped	yes

For explanation see text.

proximal segment of chromosome II attached to and ending in the X heterochromatin, the other composed of practically the entire X attached to a distal segment of chromosome II (see Figures 4, 4a, 5, and 5a). The former chromosome, of course, should be a tiny rod at metaphase and possess the centromere obtained from II; the latter should be a somewhat unequal V and possess the centromere obtained from the X.

That this is exactly what was found can be seen by studying the photomicrographs shown in Figures 6, 8, and 9. By way of comparison the normal chromosome group at second metaphase is shown in Figure 7. The cells in this figure show very clearly a rod-shaped "precocious" chromosome at one pole and five chromosomes on the plate. Of the five, three are members of the regular complement and two are V-shaped "limited" chromosomes. The limited chromosomes are supernumerary and occur only in the germ line; they are eliminated from the somatic nuclei of the embryo at the fifth or at the sixth cleavage (Du Bois 1933). In some species of *Sciara* limited chromosomes do not occur. Their occurrence in *S. coprophila* is of no significance for the problems under consideration in this paper. The spermatocytes from the OR T1 testis (Figures 6, 8, and 9) reveal a complement of four ordinary and three limited chromosomes (the number of limited chromosomes is variable in this species). All of the limited chromosomes are V-shaped. In the polar view of second metaphase shown in Figure 6 the four ordinary chromosomes lie adjacent to each other in the lower half of the nucleus, the three limited ones roughly in the upper half; the chromosomes at this stage are clearly double, and the tiny rod looks superficially like a small V; of the remaining three ordinary chromosomes only one is a rod, the other two being V-shaped. In other words, by means of translocation OR T1 a normal complement of three rods and one V was converted into two rods and two V's (see above). That this is clearly the case can be seen in the anaphase in Figure 9 taken from the same testis; the rod-shaped "precocious" chromosome is at the upper pole and looks somewhat like a triangle whose apex is pointing

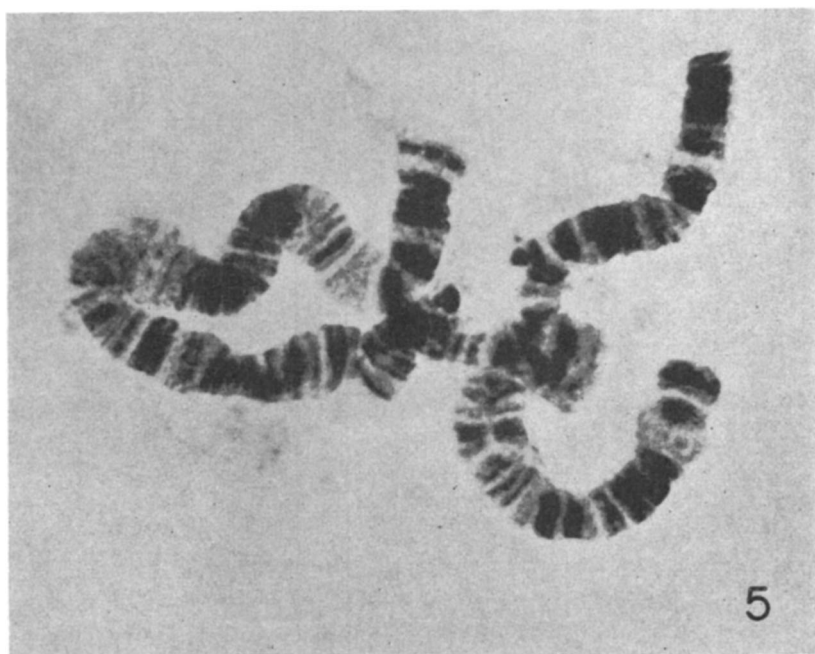
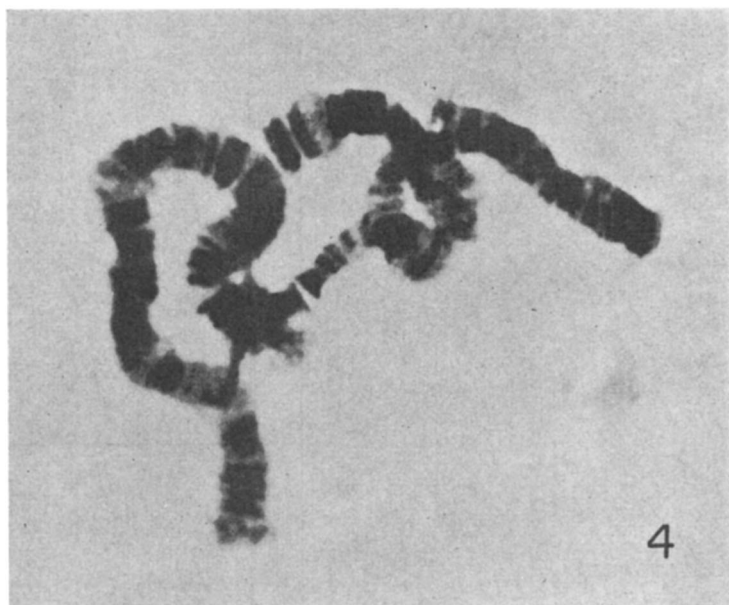


FIGURE 4.—Photomicrograph of heterozygous OR T1 showing lateral synapsis of the three repeat regions. $\times 1500$.

FIGURE 5.—Photomicrograph of heterozygous OR T1. In this nucleus the interstitial repeat is not synapsed with the other two. $\times 1500$.

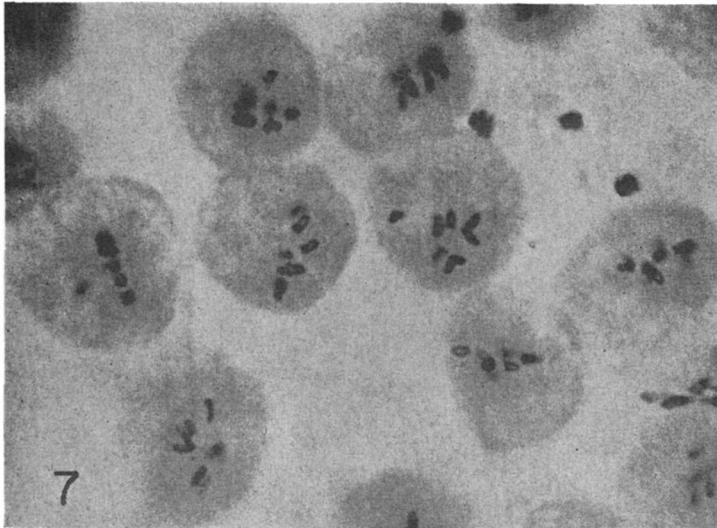
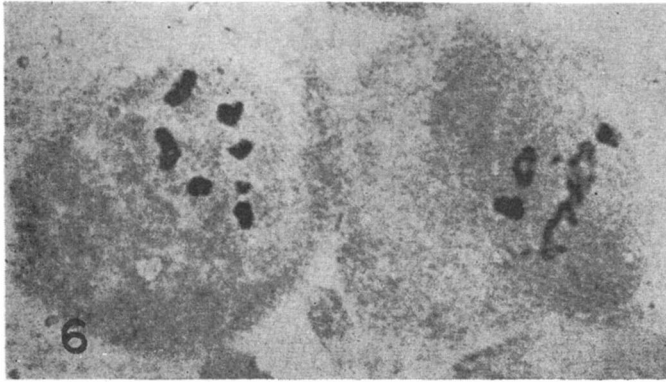


FIGURE 6.—Orcein squash of secondary spermatocytes of OR T1. Photomicrograph at 1500 \times . Polar view of M II; in the A II figure the tiny rod chromosome is dividing equationally (see text).

FIGURE 7.—Secondary spermatocytes of PT 1 showing a chromosome complement which is indistinguishable from that observed in normal material at this stage. Note the rod-like precocious chromosome and the two other rods on the equatorial plate (see text).

toward the equatorial plate; all the chromosomes undergoing division on the plate are V-shaped except one; this rod is III. In orcein squashes it is difficult to determine, in polar view of second metaphase, which chromosome is exhibiting precocity, e.g., the one in Figure 6; in side view, however, the "precocious" chromosome can be recognized readily, and in all four side views in Figure 8 as well as in the one in Figure 9, the tiny rod is behaving precociously. In all these figures this chromosome is pale and attenuated and definitely oriented with its apex towards the plate. Which end of the rod bears the centromere of II and

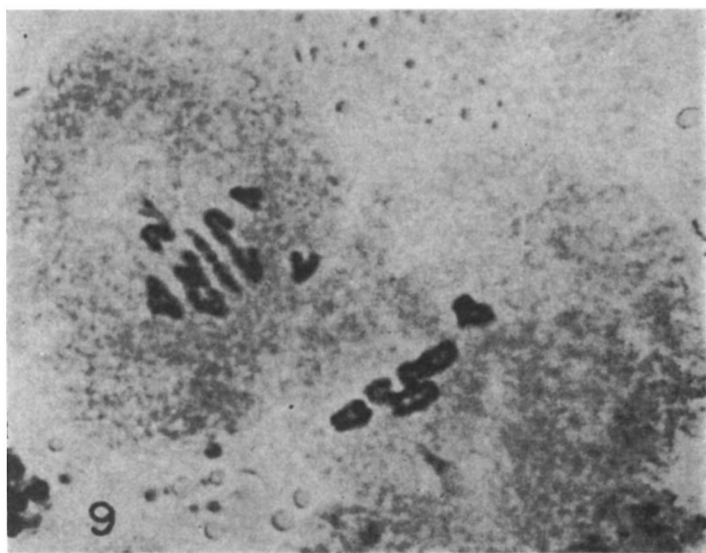
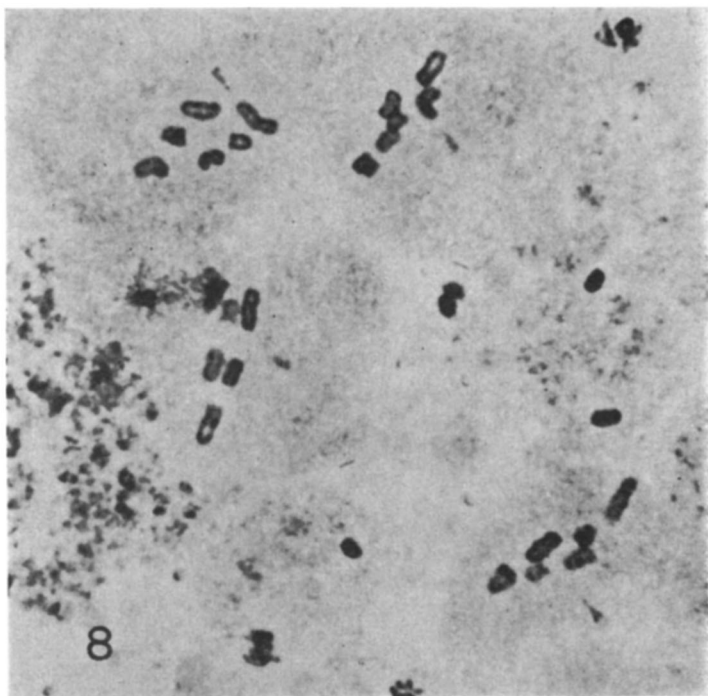


FIGURE 8.—Same testis as shown in Figure 6. Side views of M II in which precocious behavior of tiny rod can be clearly seen. $\times 1500$.

FIGURE 9.—Same testis as shown in Figures 6 and 8. Side view of M II; A II figure shows precocious tiny rod towards upper pole. $\times 1500$.

which the heterochromatin from the X is not certain. Very often in orcein squashes of normal material, particularly in *S. reynoldsi*, the two chromatids of the precocious chromosome diverge distally as they go to the pole. Reasoning by analogy, then, one would be led to believe that the centromere of the tiny rod in the OR T1 spermatocytes actually backs into the pole and that the end of this translocation chromosome which is made up of the X heterochromatin actually leads the way. Whether this is indeed the case has not yet been determined from the sectioned material; it is hoped that with osmium fixation, better presentation of the spindle fibers will be obtained.

The anaphase shown in Figure 6 is very interesting because the tiny rod has failed to exhibit precocity in this spermatocyte, and, instead, has clearly undergone equational division. The fate of such cells subsequent to second anaphase is unknown. An estimate of the frequency with which this behavior occurs was made by careful study and tabulation of approximately 1000 spermatocytes at second metaphase and anaphase: in 940 cells the tiny rod clearly exhibited precocity; in eight cells it underwent equational division; and in 33 cells its behavior could not be determined. In the course of this careful tabulation no chromosome other than the tiny rod was ever found to exhibit precocity, even in those cells in which this rod underwent equational division.

By way of summary of the cytological studies on spermatogenesis, then, it can be stated that for each of the X translocations, only one of the rearranged chromosomes is transmitted in duplicate through the sperm: in every case except OR T1 it is the chromosome which is made up of a proximal segment of X, including the X centromere and terminal heterochromatin. In OR T1, where the heterochromatin and centromere have become separated from each other, it is the rearranged chromosome composed of the X heterochromatin and a proximal segment of II which is transmitted in duplicate.

Inheritance of the translocations through the male: Once the chromosome complement included in the sperm nucleus had been determined for the various translocations, it was possible to anticipate the kinds of offspring that would be derived by breeding the males to normal females. This has been done in diagrammatic fashion in Figure 11 for all of the translocations except OR T1 and in Figure 10 for OR T1. In both diagrams the chromosome eliminations from the embryonic soma and germ line are based on the assumption that the translocation chromosome undergoing equational nondisjunction during spermatogenesis is likewise the chromosome to be eliminated during development. On this basis the daughters should be heterozygous (soma and germ line) for the translocation in question. That this is indeed the case was determined by genetic study and by cytological examination of the larval salivary and oögonial complements. The genetic tests consisted of phenotypic classification of the adult females and a similar classification of their progeny (see CROUSE 1943, 1960). Although the differentiation of sons is considered as a possibility in both diagrams, actually none was found; the male embryos failed to hatch out of the eggs. Their death at an early stage of embryonic development gives support to the correctness of the scheme of chromosome elimination followed in the diagrams. Presumably the

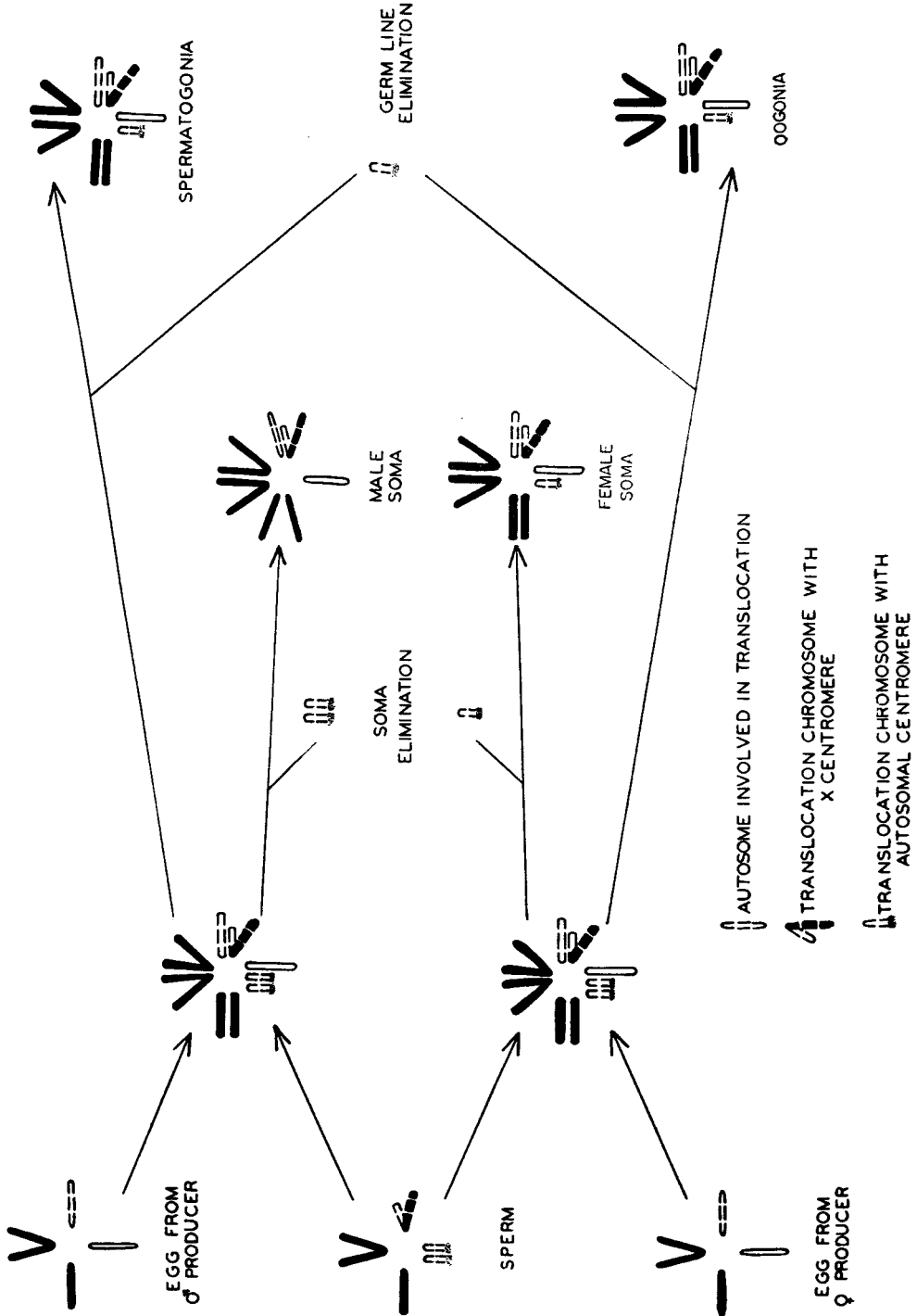


FIGURE 10.—Diagram showing chromosome history in progeny derived from OR T1 males bred to normal females.

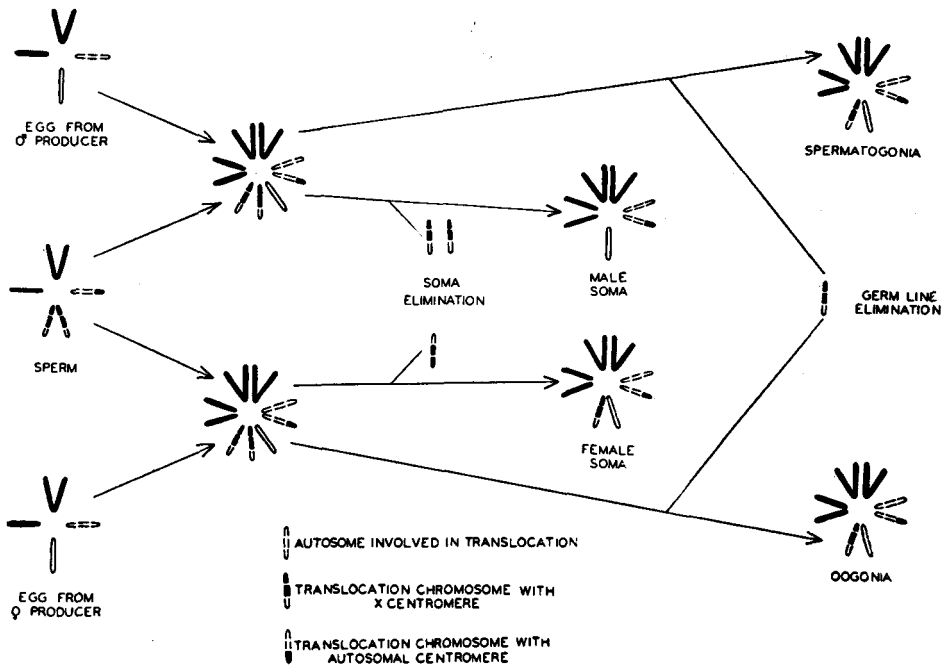


FIGURE 11.—Same as the diagram in Figure 10 except that it applies not to OR T1 but to the other eight translocations.

males die because of the unbalanced complement in their somatic nuclei: for each of the translocations the complement is deficient for a segment of autosome and duplicated for a segment of X, the X duplication in the case of OR T1 comprising all of the chromosome except the terminal mass of heterochromatin.

Inheritance of the translocations through the female: Meiosis in the *Sciara* female is perfectly orthodox and involves crossing over and the random assortment of homologues. Consequently, females heterozygous for an X translocation would be expected to form two classes of euploid eggs. That this is the case has already been established for both the Missouri (CROUSE 1943) and the Pennsylvania translocations (CROUSE 1960). It is true also of the Oak Ridge series (unpublished). In addition to the euploid eggs, aneuploid types arise through nondisjunction (CROUSE 1960). Aside from the phenomenon of nondisjunction, the X translocations are inherited in a regular, orthodox manner.

DISCUSSION

The inheritance of sex chromosomes in *Sciara* and their pattern of behavior during development constitute a unique mechanism of sex determination and provide clear demonstration that the cells can distinguish one chromosome from another. This "recognition" results in the elimination of the chromosome or chromosomes from the cell and, thereby, in an altered genetic condition. The

translocation studies show clearly that it is not the whole X chromosome which is involved in the elaborate series of interrelated cytological events but only a very localized region, namely, the terminal (proximal) mass of heterochromatin. This region controls both equational nondisjunction in the secondary spermatocyte and chromosome elimination in the embryo; without it the sex chromosome behaves like an autosome, but with it the autosome behaves like X.

That the controlling region proves to be the heterochromatin which normally lies adjacent to the X centromere is, in the last analysis, probably not surprising. In view of the *Sciara* data one might have chosen the X centromere as the most plausible controlling element. First, the dramatic chromosome unorthodoxies in *Sciara* are clearly unrelated to the genic make-up of the chromosomes: a chromosome which passes through the male germ line acquires an "imprint" which will result in behavior exactly opposite to the "imprint" conferred on the same chromosome by the female germ line. In other words, the "imprint" a chromosome bears is unrelated to the genic constitution of the chromosome and is determined only by the sex of the germ line through which the chromosome has been inherited. Second, centric activity is obviously involved in equational nondisjunction, in chromosome elimination at anaphase (embryonic soma), and probably it is involved also in the prochromosome elimination from the germ cells at resting stage. That the controlling element on the X is not the centromere but an element which lies in the adjacent heterochromatin is in line with and gives support to the view advocated by NOVITSKI (1955) and by LINDSLEY and NOVITSKI (1958), that the kinetic activity of centromeres in *Drosophila* (as measured in anaphase bridge experiments) is to be attributed to the constitution of the heterochromatin immediately adjacent to the centromeres. That the heterochromatin of the *Sciara* X, upon translocation (OR T1) to the proximal segment of chromosome II, can control the behavior of the rearranged chromosome is indeed very interesting. Not only is the centromere considerably removed (opposite end of the rearranged chromosome) from the translocated heterochromatin, but it is a non-homologous centromere, the one derived from chromosome II. This result supports the view advocated by LINDSLEY and NOVITSKI (1958), that the function of the centromere is constant from chromosome to chromosome.

From the translocation studies it appears that a balanced chromosome complement is not essential in the early stages of *Sciara* development. The zygote can be either hypoploid or aneuploid and still give rise to an adult organism, provided a euploid XX or XO somatic complement is acquired at the time of elimination. Thus zygotes which, as a result of nondisjunction, receive no sex chromosome from the egg nucleus, develop under the influence of two sex chromosomes (paternal) instead of three and become exceptional (patroclinous) males among the progeny of female-producing mothers (CROUSE 1960). And in the case of each X translocation taken through the male germ line, daughters are derived from aneuploid zygotes which carry an autosomal duplication and a deficiency on the X; in the case of OR T1 the deficiency includes all of the X chromosome except the terminal heterochromatin.

SUMMARY

Nine reciprocal translocations between chromosome X and the autosomes of *S. coprophila* have been studied in relation to their effects on spermatogenesis and chromosome elimination in the embryo. In every case one of the translocation chromosomes displayed behavior typical of the autosomes at the second spermatocyte division; the other exhibited precocity and thus underwent equational nondisjunction. The chromosome eliminated later from the embryonic soma and germ line was the translocation chromosome which had undergone equational nondisjunction during spermatogenesis. Precocity at the second spermatocyte division and elimination at the critical stages in the embryo were found to be under the control of the terminal heterochromatin which is located normally adjacent to the X centromere. When, by reciprocal translocation, this mass of heterochromatin comes to form the distal end of a rearranged chromosome which bears the proximal segment of II, it controls the behavior of this chromosome in spite of its wide separation from the centromere.

LITERATURE CITED

- BERRY, R. O., 1941 Chromosome behavior in the germ cells and development of the gonads of *Sciara ocellaris*. *J. Morphol.* **68**: 547-583.
- CARSON, H. L., 1946 The selective elimination of inversion dicentric chromatids during meiosis in the eggs of *Sciara impatiens*. *Genetics* **31**: 95-113.
- CROUSE, H. V., 1943 Translocations in *Sciara*; their bearing on chromosome behavior and sex determination. *Missouri Univ. Research Bull.* **379**: 1-75.
- 1960 The nature of the influence of X translocations on sex of progeny in *Sciara coprophila*. *Chromosoma* **11**: 146-166.
- DUBOIS, A. M., 1933 Chromosome behavior during cleavage in the eggs of *Sciara coprophila* in relation to the problem of sex determination. *Z. Zellforsch.* **19**: 595-614.
- LINDSLEY, D. L., and E. NOVITSKI, 1958 Localization of the genetic factors responsible for the kinetic activity of X chromosomes of *Drosophila melanogaster*. *Genetics* **43**: 790-798.
- METZ, C. W., 1938 Chromosome behavior, inheritance, and sex determination in *Sciara*. *Am. Naturalist* **72**: 485-520.
- NOVITSKI, E., 1955 Genetic measures of centromere activity in *Drosophila melanogaster*. *J. Cellular Comp. Physiol.* **45** (suppl. 2): 151-169.